LOOKING AHEAD

Preclinical pharmacology indicates strongly that p38 inhibitors are likely to be efficacious in the treatment of human rheumatoid arthritis. Ongoing clinical studies on at least two structurally different p38 inhibitors should reveal whether efficacy can be demonstrated at tolerated doses.

Potential of p38 Inhibitors in the Treatment of Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology. Anatomically it affects mainly, but not exclusively, the synovial joints. Patients presenting with established RA suffer a range of extraarticular syndromes that significantly contribute to the morbidity and mortality that are a feature of this condition. Within the synovial joints of patients with RA, chronic (lymphocyte-mediated) and active chronic (lymphocyte- or neutrophil-mediated) tissue inflammation are the major mechanisms leading to tissue damage. RA is a polyarthropathy affecting most commonly synovial or diarthrodial joints.[1]

Summary
Rheumatoid arthritis (RA) is a chronic debilitating disease estimated to afflict 1–3% of the world population. Although palliative treatments (nonsteroidal anti-Inflammatory drugs or NSAIDs) are widely prescribed, there are currently only a few treatments that can modify the insidious progression of the disease (disease-modifying antirheumatic drugs or DMARDs), which frequently leads to physical incapacitation and, on occasion, death. Proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) are implicated in the disease onset and in the progression of bone and joint destruction that characterizes chronic RA. p38 is an intracellular mitogen/stress-activated protein kinase (MAPK/SAPK) that regulates both the release and the actions of TNF-α and IL-1β. Inhibition of p38 kinase is thus an important potential target for novel DMARDs. Following the pioneering work conducted at SmithKline Beecham and elucidation of the roles of p38 with potent and selective inhibitors such as SB-203580, many pharmaceutical companies have embarked upon p38 synthetic programs, as indicated by the ever-increasing number of patents in this domain. At Aventis, a rapid parallel synthesis project led to the identification of RPR-200765A, a potent and selective p38 inhibitor of the lipopolysaccharide-induced release of TNF-α *in vitro* in mononuclear phagocytes and *in vivo* in the rat. It also reduces disease incidence and progression in the rat streptococcal cell wall arthritis model when administered orally in either a prophylactic or a therapeutic dosing regimen. Development of p38 inhibitors has been slow, probably because of toxicological problems, which might explain why only two oral p38 inhibitors, SB-242235 and VX-745, have advanced into clinical development. In the present article, the preclinical data exemplified in studies on RPR-200765A indicating why p38 inhibitors are attracting so much attention as potential novel anti-RA drugs are reviewed. Current information on the different structural classes of p38 inhibitors is presented and possible reasons for the delays in their development are critically discussed. © 2000 Prous Science. All rights reserved.

The development of RA pathology in the synovial joints may be divided into three phases: the acute phase, characterized by hyperemia, edema and a neutrophil-rich infiltrate; the chronic phase, characterized by a largely lymphocytic cell infiltrate, tissue damage and fibrosis; and the end-stage lesion, characterized by postinflammatory fibrosis, scarring and secondary events (e.g., osteopenia and osteoarthritis). Histopathological studies show these phases are not mutually exclusive, and disease progression at any one time point may embrace facets of two or more phases or show transitional stages between these phases. The anatomy of the end-stage lesion commonly features postinflammatory osteoporosis.[1]

Although the cellular pathology of RA is complex, there can be little doubt about the key contributions of the lymphocyte and monocyte axes to the initiation and development of the lesion. The cardinal histopathological feature of RA is synovial hyperplasia with a predominance of T cells within the synovial villi. T cells constitute around 20–50% of cells recovered from the rheumatoid synovium. Monocytes are also abundant, with about 30–50% of the population actively presenting antigen during the disease.[1]

Although T cells are the dominant cell type, T cell-derived cytokines such as interleukin-2 (IL-2), IL-4 and interferon gamma are either low or undetectable in tissues derived from RA patients. In contrast, it is monocyte-derived cytokines which characterize the molecular pathology of the RA lesion. The major cytokines found in disease tissues are IL-1β, IL-6 and tumor necrosis factor-α (TNF-α), in addition to the pleiotropic growth factors, such as platelet-derived growth factor (PDGF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and transforming growth factor-β (TGF-β). There is now convincing evidence that these molecules are key orchestrators of the tissue pathology seen in RA, especially in relation to synovial hyperplasia, pannus infiltration and bone resorption.[2] Indeed,

experimental administration of TNF-α alone elicits synovitis.[3]

Current therapy

Rheumatoid arthritis remains a condition in which there is a high unmet medical need for new therapeutics. Polypharmacy is common in this disease and, in active phases, dose levels and frequency are often high. First-line therapy is, and will probably remain for the foreseeable future, directed toward alleviation of pain. The main therapeutic class in this area comprises the NSAIDs which, although effective in palliative care, have little impact upon long-term disease progression or severity. Even drugs from the latest generation of NSAID therapy, the cyclooxygenase-2 (COX-2) inhibitors, appear to reduce symptoms with an improved therapeutic ratio (fewer gastrointestinal side effects), but with equivocal impact upon tissue pathology.

Second-line therapy is exemplified by the disease-modifying antirheumatic drugs (DMARDs). These compounds have their advocates, but suffer from lack of appropriate clinical trial assessment with consequent poor standardization of dose regimens. Therapeutic ratio is a problem with this class of agent, possibly owing to the often very high doses used, which results in patients being maintained on these compounds for very variable amounts of time as a result of intolerance. This contributes to the lack of rigorous data for these drugs. The archetypal DMARD that dominates the clinical pharmacology of this area is methotrexate; however, as with others of the class, therapeutic benefit is often paralleled by a significant range of severe adverse reactions.[4]

As with all inflammatory conditions, steroids have a ubiquitous presence in the therapy of RA. In some trials, prednisolone has shown beneficial impact upon both symptom severity and radiological progression of the disease. These trials have become paradigms for antirheumatic drug evaluation, although the work has been criticized for methodological short-

comings.[5] The major issue with chronic dosing of steroids is the consequence of their impact upon the HPA axis, which can induce osteopenia and further weaken an already compromised rheumatoid joint. Recent data do show an association between steroid use and increased fracture risk in RA patients.[5]

A therapeutic area that is attracting increased focus in the use of biologicals, such as soluble TNF-α and IL-1β receptors, in addition to anti-TNF-α antibodies. Early development of these agents revealed them to be dose-limited owing to immunological adverse reactions. However, the development of strategies for the humanization of nonhuman proteins has transformed this approach, and several trials have shown beneficial effects on both symptom severity and radiological progression.[6]

In summary, the field of rheumatoid arthritis therapeutics is still awaiting effective, disease-modifying agents with acceptable therapeutic ratios. Although the biologicals area shows much promise, the need for orally active and safe low-molecular-weight molecules is still great.

p38 Kinase: Potential target for novel anti-RA drugs

Much effort is currently being directed toward targeting protein kinases to discover novel antiinflammatory drugs.[7,8] Since mitogen-activated protein kinase (MAPK) pathways have been implicated in several chronic antiinflammatory disorders,[9] they are particularly attractive targets for drug intervention. Three major MAPK pathways—extracellular regulated kinase (ERK), cJun N-terminal kinase (JNK) and p38—exist in eukaryotic cells which, via complex phosphorylation cascades, transduce extracellular signals to intracellular responses.[10] In addition to proinflammatory agents such as LPS, TNF-α and IL-1β, JNK and p38 are activated by agents (ultraviolet light, heat, osmotic stress, anisomycin) that cause cellular stress and are therefore also known as stress-activated protein kinases (JNK-SAPK1; p38-SAPK2).[10] Several iso-
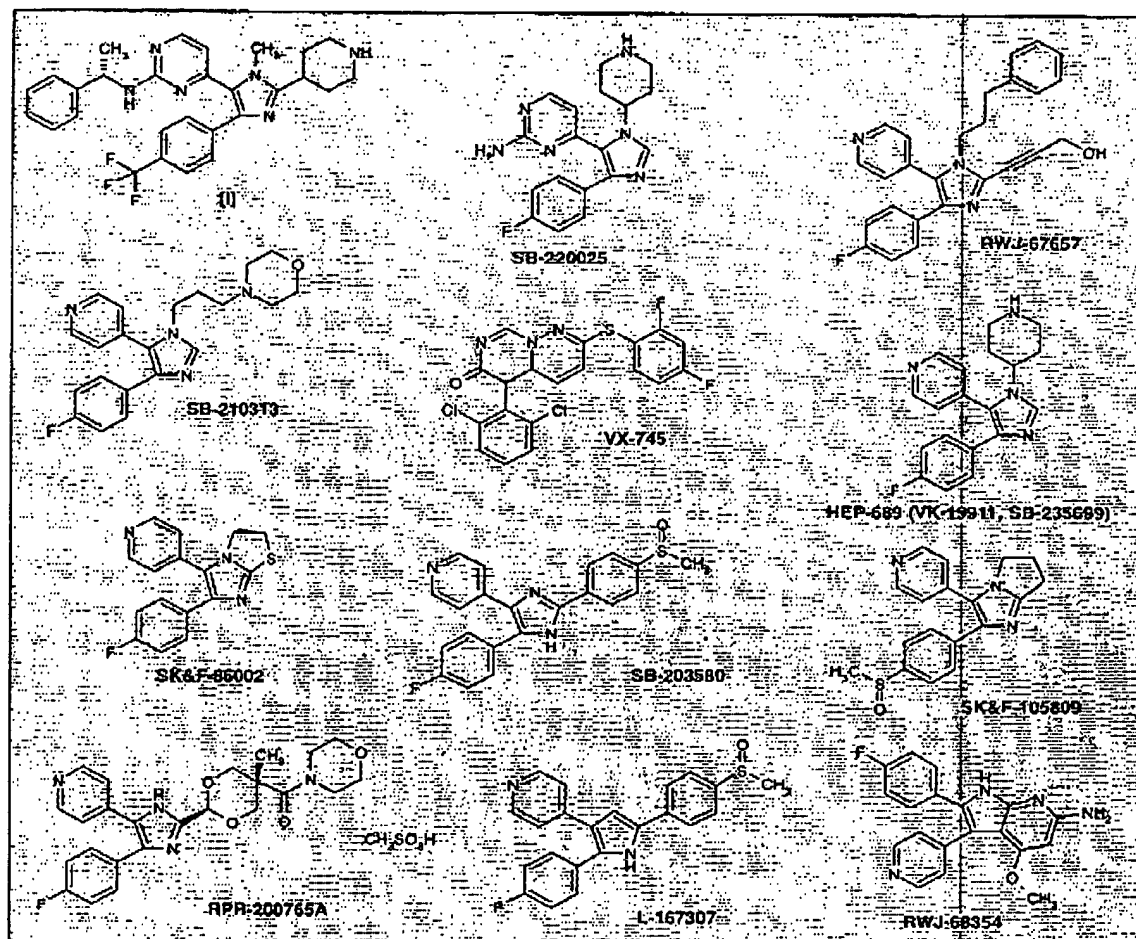
Fig. 1. Structures of p38 kinase inhibitors under investigation.

forms of each MAPK/SAPK exist and four homologues of p38 (p38α, β, γ and δ) have been identified which, although activated by stress and inflammatory stimuli, are thought to play different intracellular roles.[11,12]

Elegant studies performed at SmithKline Beecham were critical in demonstrating the importance of p38 as a therapeutic target for novel antiinflammatory drugs and provided tools that have been invaluable in dissecting the roles of this SAPK in diverse biological systems. A breakthrough came with the demonstration that certain members of a series of bicyclic 2,3

dihydroimidazo[2,1-b]thiazoles, originally described as dual lipoxygenase (LO)/cyclooxygenase (COX) inhibitors, suppress LPS-induced TNF-α and IL-1β release from human monocytes.[13] The mechanism by which pyridinylimidazoles inhibit cytokine release was reported in a seminal paper in 1994.[14] By use of the tritiated ([³H]-SB-202190) and photoaffinity ([¹²⁵I]-SB-206718) probes, the proteins to which these compounds bind were identified, partially sequenced and eventually cloned and expressed. Two recombinant proteins were shown to be human homologues of the murine p38, and called CSB-P kinase (for cytokine

suppressive antiinflammatory drug binding protein kinase).[14]

Pyridinylimidazoles, such as the archetypal SB-203580 (Fig. 1), are ATP-competitive inhibitors of p38 kinase.[15] In view of the millimolar intracellular concentrations of ATP against which kinase inhibitors presumably have to compete, it is surprising that potencies of SB-203580 on functional cellular responses are similar to its kinase activity.[4,16] The high K_m of p38 for ATP (K_m = 200 μM)[15] may offer a potential explanation for the lack of drop-off in potency in intact cells. Alternatively, SB-203580, which
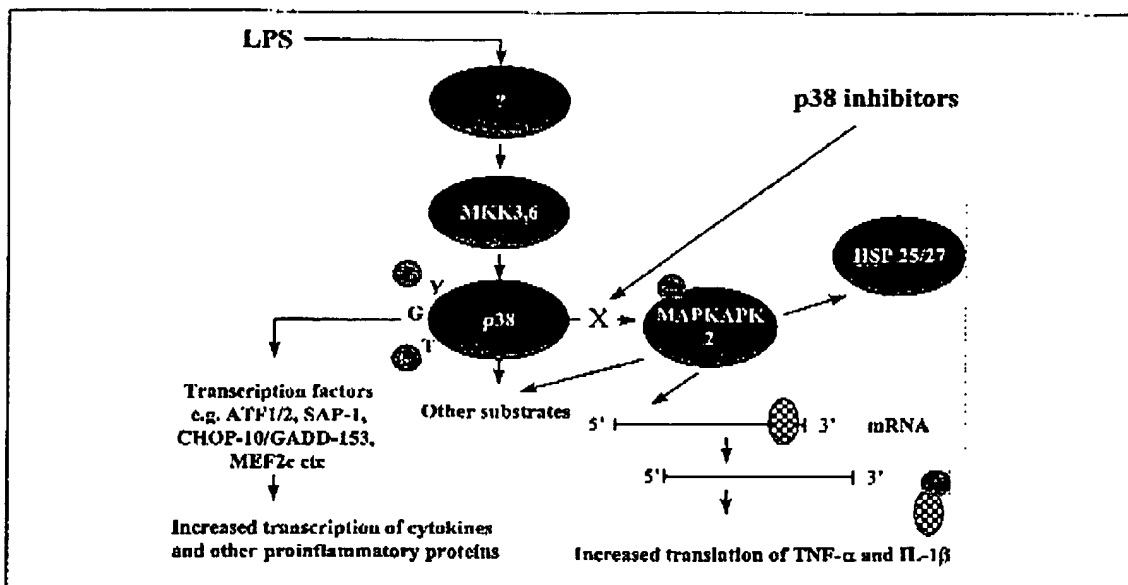
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Fig. 2. Drawing depicting the mechanism by which p38 inhibitors suppress the release of TNF-α and IL-1β. Although p38 inhibitors suppress the transcription of certain cytokines (e.g., TNF-α-induced IL-6 release), possibly through actions on transcription factors, an effect on translation is the major mechanism by which LPS-induced release of TNF-α and IL-1β from monocytes/macrophages is suppressed. p38 is proposed to phosphorylate and activate MAPKAP kinase 2, which directly or indirectly phosphorylates regulatory elements that bind to the AU-rich 3′-untranslated region of the mRNA for TNF-α and IL-1β, causing it/them to dissociate, so removing the translational repression.

does not compete with ATP for binding when the enzyme is in the inactivated state, blocks agonist-induced phosphorylation of p38α, indicating that activation, as well as activity, is inhibited by this drug.[17] However, this explanation has recently been questioned.[18] In general, pyridinylimidazoles, such as SB-203580, are selective for p38, although inhibition of certain JNK forms (JNK-2α1), Lck and c-Raf has been reported.[19] They inhibit two of the p38 isoforms (α and β), but are inactive against the other two (γ and δ).[11,19] X-ray crystallography and molecular modeling, as well as molecular biological and biochemical studies, have provided a structural basis for the selectivity of pyridinylimidazoles for p38 (see below).[20-23]

SB-203580 has been widely used to dissect the roles of p38 in cellular systems and has been shown to suppress functional responses of several inflammatory/immunocompetent cell-types.[16,24,25] The therapeutic potential

of p38 inhibitors in chronic inflammatory disorders such as RA is suggested by their suppression of the release and actions of proinflammatory cytokines, notably TNF-α and IL-1β, from mononuclear phagocytes.[14,16] Effects on cytokine mRNA stability and transcription—p38 directly or indirectly phosphorylates and transactivates several transcription factors including ATF1/2 (activating transcription factors 1/2), CHOP-10/GADD-153 (growth arrest and DNA damage inducible gene 153), SAP-1 (serum response factor accessory protein-1) and MEF2C (myocyte enhancer factor-2)—accounts for some of the antiinflammatory effects of pyridinylimidazoles.[9,10,26] However, the major mechanism by which TNF-α release from monocytes is suppressed is post-transcriptional and is most likely a result of the blockade of p38-induced enhancement of translation mediated via MAPKAP kinase 2 (for MAPK activated protein kinase) through regulatory elements that bind to the AU-

rich 3′-untranslated region of the mRNA for TNF-α (Fig. 2).[16,27,28]

In vivo, SB-203580 and other pyridinylimidazoles exhibit a profile of activities concomitant with potential for the treatment of RA and other chronic inflammatory disorders. Oral efficacy in several preclinical RA models has been reported and, importantly, conservation of joint integrity and protection of bone, cartilage and soft tissues has been observed.[29-31] Suppression of inflammation-driven angiogenesis, which supports the destructive proliferation of inflammatory tissue in the arthritic joint, may contribute to the anti-RA activity of p38 inhibitors.[22] It is likely that inhibition of inflammatory cytokine release in joints underpins these effects. In addition to their actions in RA models, efficacy of p38 inhibitors in preclinical models of endotoxic shock,[29] antigen-induced airway inflammation[33] and stroke[34] has been reported and potential of these compounds in skin disorders is currently being evaluated clinically.[19]

Chemistry of p38 inhibitors

The p38 inhibitors originate from a 5-LO and COX project at SmithKline Beecham. The two development candidates, the bicyclic imidazoles SK&F-86002 (Fig. 1) and SK&F-105809 (Fig. 1), were modified to the more potent 2,4,5-triarylimidazole SB-203580 and analogues.[25,35]

Since the report in 1994 identifying p38 inhibition as the mechanism by which these compounds suppress TNF-α release,[14] several pharmaceutical companies have entered the field, and synthesis of structurally related and unrelated analogues has been the subject of many patents and papers, which have been reviewed extensively.[8,19,36,38]

Crystallographic data on SB-203580 bound to p38 have revealed that this ATP competitive inhibitor mainly exhibits two important binding features.[39] Firstly, the 4-pyridyl moiety mimics the adenine ring of ATP and forms a hydrogen bond interaction with the main chain NH of Met[109]. Substitution of the 4-pyridyl moiety with a pyrimidine or an aminopyrimidine has been shown to retain the hydrogen bonding ability (see below, e.g., SB-220025; Fig. 1) and also to minimize CYP interaction (see below). Secondly the 4-fluorophenyl group fits tightly in a hydrophobic pocket containing the Thr[106] and Leu[104] residues and contributes to the binding and the selectivity to p38.

The central imidazole ring, although not essential, adds to the binding, forming a hydrogen bond with the amino group of Lys[53,39] This 4-aryl-5-pyridinylimidazole can be considered the pharmacophore; indeed, the simple compound, 4-(4-fluorophenyl)-5-(4-pyridinyl)imidazole, has been reported to have a good p38 inhibitory activity (IC_{50} = 210 nM).[40] Nevertheless, it has been shown that a pyrrole ring can replace the imidazole, as in L-167307 (Fig. 1), leading to increased potency,[41] although modeling of L-167307 did not show the formation of a favorable interaction with Lys[53] as

seen with imidazole-containing inhibitors.[21]

The 4-methylsulfinylphenyl group of SB-203580 contributes to the binding through a stacking interaction with Tyr[35].[39] Substituents at position C-2 of the imidazole ring, which bind in the ATP phosphate-binding region, are not critical for binding and can be replaced with other groups exhibiting a new favorable interaction. For example, this can be seen with VK-19911 (HEP-689, SB-235699; Fig. 1), which lacks a C-2 substituent while having a 4-piperidine ring at position N-1, forming a salt bridge with Asp[168].[42] The same feature has been reported for SB-220025, a closely related aminopyrimidine analogue.[39]

Most of the p38 inhibitors have been constructed around the 4-aryl-5-pyridinylimidazole pharmacophore,[19,38] varying the substituents at N-1 and C-2, as shown in SB-210313 (Fig. 1),[40] VK-19911,[37] RWJ-67657 (Fig. 1),[43] a recent series of very potent and selective imidazole inhibitors ([II], Fig. 1) from Merck[44] and RPR-200765A (Fig. 1).[31] to name a few. Other modifications have involved the imidazole ring replacement with different 5- and 6-membered heterocycles such as oxazole, pyrazole, pyrimidine,[19,38] furan, pyrazolone or indole as, for example, in the above-mentioned L-167307,[41] or fused pyrrolo[2,3-b]pyridines as in RWJ-68354 (Fig. 1).[45,46]

Recently, new structures unrelated to the 4-aryl-5-pyridinylimidazole pharmacophore that were probably identified by drug design or high-throughput screening against p38 kinase have started to emerge. These include the Vertex series of diaryl-triazanaphthalenones[47] related to VX-745 (Fig. 1)[19] or indoles, indolones, ureas[19] and benzanilides related to the low density lipoprotein (LDL) receptor up-regulator RPR-102359[48,49] and, more recently, benzylpiperidinones and benzylpiperazinones.[50]

RPR-200765A,[31,51,52] a 2-dioxolane 4-aryl-5-pyridinylimidazole,

was designed as a structural hybrid of SB-203580 and a former series of bioavailable and systemically stable 2-dioxolane 4,5-diphenylimidazole ACAT inhibitors.[53] Modeling of RPR-200765 in the ATP site of p38 has shown a favorable binding interaction of the morpholino group with Lys[152] (Fig. 3).

Pharmacology of RPR-200765A

RPR-200765A is a potent, selective ATP-competitive inhibitor of p38 (K_i = 50 nM) that suppresses TNF-α release from human peripheral blood monocytes (EC_{50} = 110 nM) with a potency broadly equivalent to that of the standard SB-203580 (EC_{50} = 146 nM).

The in vivo pharmacology of RPR-200765A reveals significant activity in both acute mechanistic models of TNF-α release. In addition to significant disease-modifying activity in a rodent model of joint inflammation. Administration of LPS to rats induces an elevation in peripheral blood TNF-α that is significantly attenuated by oral administration of RPR-200765A with a potency (ED_{50} = 3–10 mg/kg) broadly equivalent to that of SB-203580 under the same dosing conditions (Fig. 4).

We have previously shown that both TNF-α and IL-1β are highly expressed in the hyperplastic synovium of rats following streptococcal cell wall (SCW) insult.[7,44] This, together with previous data showing that compounds impacting these cytokine axes have efficacy in this model, supports its selection for p38 compound screening. The administration of SCW extract to susceptible rat strains initiates a range of systemic organ pathologies, most notably arthritis.[8,55] Within 24 hours after intraperitoneal injection of SCW, the animals develop a symmetrical synovitis of distal joints that matures to yield a chronic synovitis, inflammatory cell infiltrates into the joint and synovial villus hypertrophy. Later, a range of end-stage lesions are apparent, such as pannus, bone erosions and fibrosis.
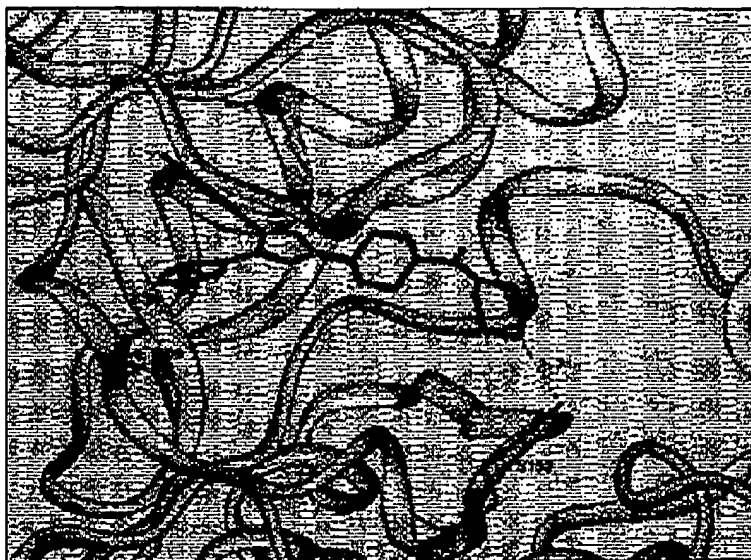
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Fig. 3. Docking of RPR-200765 in p38 ATP site showed that the morpholino group binds to Lys[1+2], the pyridine and imidazole being within H-bond distance to Met[109] and Lys[53], respectively (the distance between heavy atoms is in angstroms).
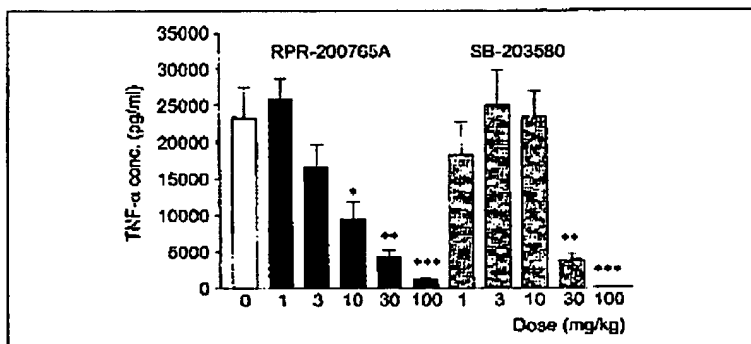

Fig. 4. Suppression of LPS-induced TNF-α release into the serum of rats by RPR-200765A and SB-203580.

The arthritic reaction to SCW may be synchronized by a sensitization phase in which an intraarticular injection of SCW is carried out. This creates a low-grade synovial lesion that may be reactivated by a later intravenous challenge with the same extract. In our studies, we have assessed key outcomes such as paw edema and radiography, but have also developed morphometric analyses to quantify intact (i.e., non-disease-involved) joint area, area of synovial hyperplasia, pannus area (i.e., area of the invasive hyperplastic synovium that impacts on bone) and bone resorption index (as a marker of osteopenia).

RPR-200765A induced significant antiinflammatory effects when administered in a prophylactic (i.e., before SCW challenge) regimen in the SCW model. The compound elicited significant depression of paw swelling, radiographic severity of the lesion and histopathology, with significant effects

being apparent at 10–30 mg/kg/day dosing. Histopathology changes were quantified for key lesions using histomorphometry and the results are presented below. SB-203580 had a similar effect on paw swelling and radiology score. The positive control, dexamethasone (0.5 mg/kg), completely prevented the reactivation of ankle swelling following SCW challenge and produced a significant reduction in radiographic pathology (data not shown).

As well as reducing joint swelling and radiographic pathology, histopathological analysis revealed that RPR-200765A preserved joint area and reduced synovial hyperplasia, pannus formation and the resorption index. Significant inhibition of three of these histopathological parameters was observed at doses of 10 mg/kg, with the remaining parameter (resorption index) being suppressed at 30 mg/kg (Fig. 5).

These data show that RPR-200765A can effectively inhibit the development of inflammatory arthropathy with impact upon both acute inflammatory edema and reactive tissue pathology. The impact of RPR-200765A on the development of paw edema and joint pathology was also studied in a therapeutic regimen. Following intravenous injection with SCW on day 21, the compounds were administered from days 23 to 27. RPR-200765A reduced edema in a dose-related manner, with significant effects being observed at 10 and 30 mg/kg (Fig. 6).

Radiographic analysis at study termination (day 28) showed that RPR-200765A, administered therapeutically, suppresses total joint pathology over a dose range similar to that observed in the prophylactic regimen. Dose-dependent inhibition of all histopathological parameters was seen for RPR-200765A, with significant effects observed at doses of 10 and 30 mg/kg. The effects of RPR-200765A at 30 mg/kg on radiographic and histopathology parameters were broadly
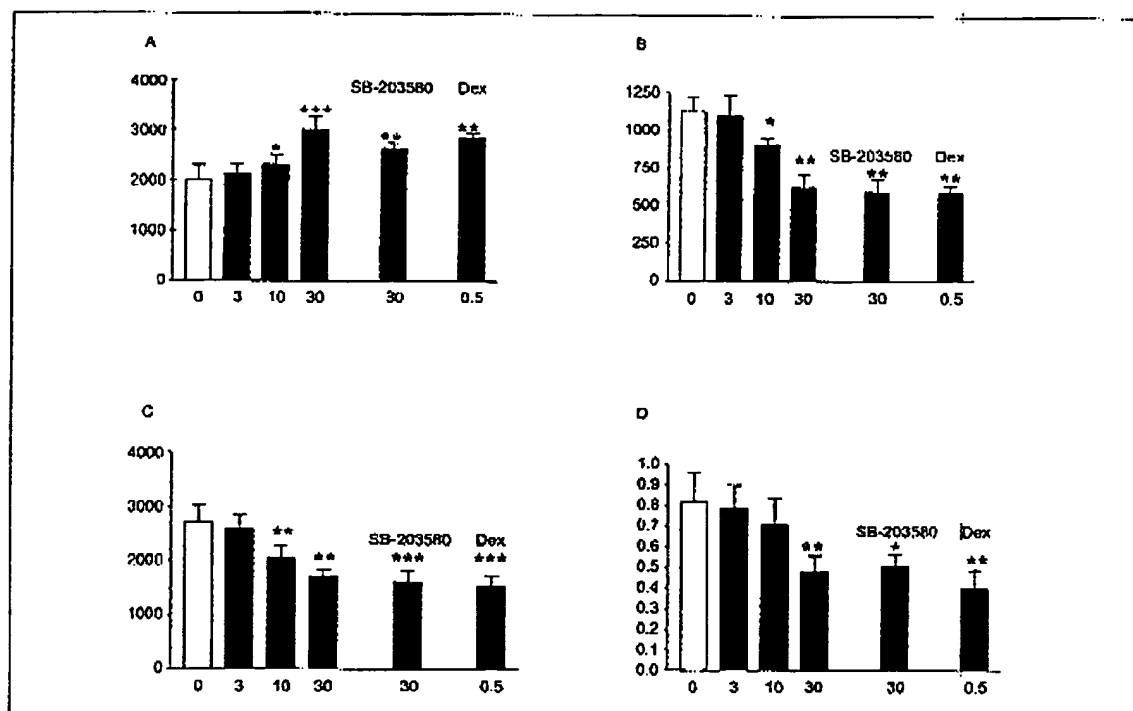
Fig. 5. Prophylactic treatment with RPR-200765A and SB-203580 in SCW arthritis in rats. Histomorphometric analysis of tibio-tarsal joints. RPR-200765A (3, 10, 30 and 100 mg/kg/day) was dosed on days 20–24, the first doses of the compound being given before reactivation of the lesion by i.v. administration of SCW extracts on day 21. SB-203580 was used as a comparison at 30 mg/kg/day and the standard dexamethasone (Dex) at 0.5 mg/kg/day. The histology was assessed in joints obtained at the study termination (day 26) and quantified in terms of joint area (panel A), pannus (panel B), synovial hyperplasia (panel C) and resorption index (panel D) by histomorphometric analysis. Results represent means ± S.E.M. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to relevant vehicle control.

equivalent to those of SB-203580 at the same dose.

In summary, these data show that RPR-200765A, when administered prophylactically or therapeutically at doses between 10 and 30 mg/kg, suppresses both paw edema and tissue pathology associated with SCW insult. Of particular note is the impact of RPR-200765A upon the bone resorption index, which suggests that this class of compound may attenuate postinflammatory osteoporosis, which is the cardinal skeletal response to RA.

Factors delaying the clinical development of p38 inhibitors

There is little doubt that p38 inhibitors exhibit a profile of activities

in preclinical RA models that if translated into humans would make them an important addition to the current armory of anti-RA drugs. In spite of this, progress has been slow, especially considering that the antiinflammatory effects of pyridinylimidazoles have been recognized for more than 10 years and the mechanism by which these compounds suppress proinflammatory cytokine release was published in 1994.[14] Although a large number of companies are active in this field, only two p38 inhibitors (VX-745 and SB-242235) have progressed into the clinic and, as yet, no phase I or phase II data have been divulged on either compound.

Undoubtedly, the development of p38 inhibitors has been hampered by

toxicological issues. This is not surprising given that currently there are no disease-modifying anti-RA low-molecular-weight compounds that are well tolerated and that the great therapeutic potential of kinase inhibitors in general has been thwarted, presumably because of unacceptable toxicities. Indeed, progress with kinase inhibitors is most advanced in cancer chemotherapy, where risk/benefit analysis is less stringent than for treatment of other diseases such as chronic inflammation. Although p38 is a widely distributed enzyme playing an important role in the functions of many cell types where interference may lead to undesirable consequences, any elucidation of mechanism-based side effects has so far not been divulged.
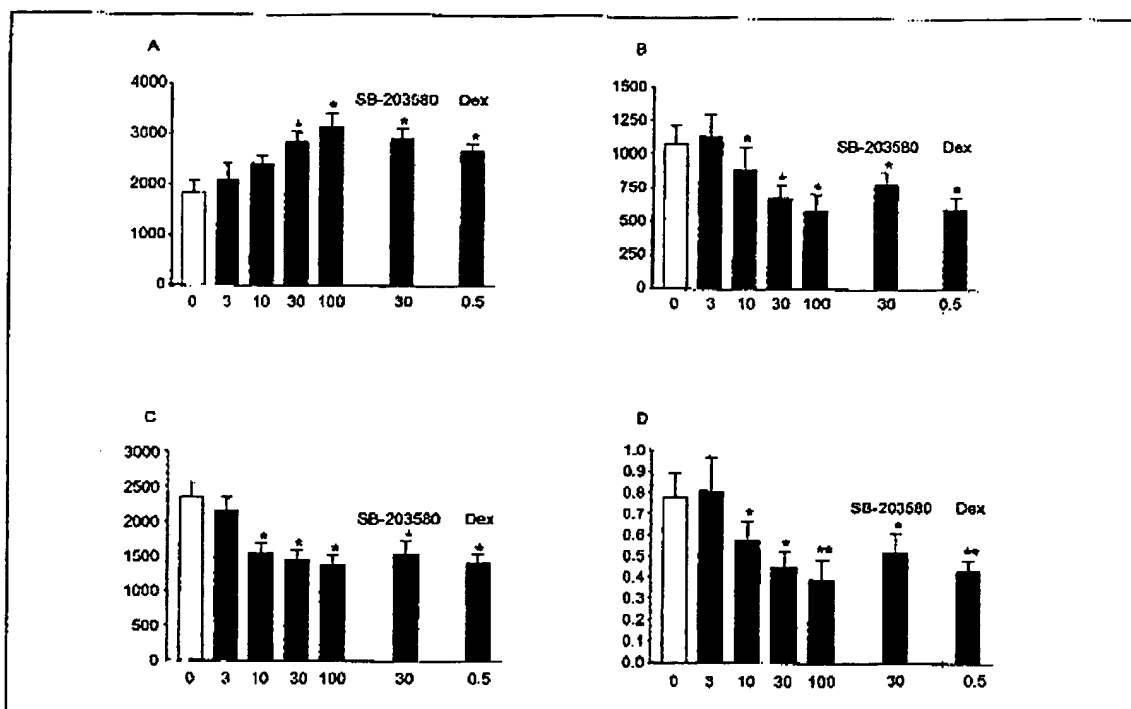
Fig. 6. Therapeutic treatment with RPR-200765A and SB-203580 in SCW arthritis in rats: Histomorphometric analysis of the tibio-tarsal joints. RPR-200765A was administered on days 23–27, the first doses of the compound being given after reactivation of the lesion by i.v. administration of SCW extracts on day 21. SB-203580 used as a comparison at 30 mg/kg/day and the standard dexamethasone (Dex) at 0.5 mg/kg/day. The histology was assessed in joints obtained at the study termination (day 28) and quantified in terms of joint area (panel A), pannus (panel B), synovial hyperplasia (panel C) and resorption index (panel D) by histomorphometric analysis. Results represent means ± S.E.M. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to relevant vehicle control.

To gain insight into p38-associated toxicities would require pharmaceutical companies to divulge their preclinical toxicological results and, obviously, this will not be immediately forthcoming. The differentiation of mechanism-based toxicities from side effects due to interactions with other kinases or nonkinase enzymes is likely to be clarified only when adverse events with pyridinylimidazoles and the novel structures are reported. However, the experience gained previously with anti-RA and immunosuppressant compounds that have progressed into human clinical trials despite exhibiting preclinical toxicity at therapeutic doses may be instructive.[16–18]

There is little information on the toxicological profile of bicyclic imida-

zoles and none on the newer, novel p38 inhibitors. SK&F-105809, an early pyridinylimidazole with dual 5-LO and COX inhibitory activity, and SB-203580 are reported to be genotoxic hepatocarcinogens.[59,60] Both compounds cause liver toxicity characterized by hepatomegaly, focal necrosis, altered foci and hepatocellular neoplasia.[60] SK&F-105809 has both initiating and promoting activities, which indicates that it is a complete hepatocarcinogen in rats.[59] It is unlikely that these toxicities are p38-related, since SK&F-105809 is a poor inhibitor of this kinase and monocyte TNF-α release,[11] although it should be noted that the sulfoxide moiety of this compound can be reductively metabolized in vivo to form the S-methyl analogue (SK&F-105561), which is a p38 inhibitor.[13]

Studies performed at Rhône-Poulenc Rorer[11] demonstrate that SK&F-105809, when administered to rats, induces an increase in liver size that is accompanied by a large elevation in the levels of cytochrome P-450s (CYP), particularly CYP1A1, the induction of which has previously been linked to hepatic neoplasia.[61] Similar CYP induction has been reported for SB-203580 and SK&F-86002.[62] Whether this effect would necessarily stop development of a compound is uncertain in view of the fact that there is at least one multi-million dollar-selling drug on the market that induces CYP1A1 and is a carcinogen in rats.[63,64] Nevertheless, elimination of CYP induction activity from pyridinylimidazoles and novel structures has been achieved.[19,41,33] The mechanism by which CYPs are induced by pyri-

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dinylimidazoles is uncertain, although the efforts made by SmithKline Beecham and others to identify compounds with markedly reduced affinity for a variety of CYPs[42] would indicate an adherence to the hypothesis that the phenomenon is an adaptive hepatic response to enzyme inhibition.[44]

There is currently no published information on other deleterious side effects of bis-arylimidazole in preclinical studies. Furthermore, it is uncertain whether the problems delaying progression of compounds are mechanism-based or structural class effects. Certainly, members of the bicyclic imidazole structural class, as well as interacting with other kinases—notably certain JNK isozymes, Lck and c-Raf[19]—may well interact with ATP-dependent and independent nonkinase enzymes, the result of which may be deleterious. Inhibitory effects of members of the pyridinylimidazole structural class on COX, LO and acyl-CoA: cholesterol acyltransferase (ACAT) have also been reported,[13,31] and it is unlikely that the list stops there. Whether the nonpyridinylimidazole p38 inhibitors are likely to be less promiscuous in their protein interactions and have a greater chance of possessing acceptable cross-species therapeutic ratios remains to be seen.

Status and future direction

There is no doubt that the preclinical pharmacology indicates strongly that p38 inhibitors are likely to be efficacious in the treatment of human RA. Elimination of TNF-α by "biological" agents is already proven as a novel therapeutic approach for RA, and orally active, low-molecular-weight compounds that suppress not only TNF-α but also IL-1β are an exciting prospect in the management of this disease. However, whether the potential becomes reality is dependent on the tolerability of pyridinylimidazoles and other structurally distinct p38 inhibitors. The next few months will be crucial, since clinical studies on at least two structurally different p38 inhibitors should reveal whether efficacy in RA can be demonstrated at tolerated doses. Whether oral therapy is a feasible option with p38 inhibitors remains to be seen. Local administra-

tion—an alternative though less attractive option—and topical application of SB-235699/HEP-689/VK-19911 are currently being clinically investigated in psoriasis.[19]

Another therapeutic target related to p38—namely, its downstream substrate, MAPKAP kinase-2—may be attractive, since murine knockout studies demonstrate a critical role in regulating TNF-α and MAPKAP^{+/−} mice have shown to be viable.[27] Before then, much effort will still be devoted to the development of p38 inhibitors and crucial information on the efficacy and therapeutic window of this class of compounds will be generated.

Acknowledgment

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